DNA compaction by the bacteriophage protein Cox studied on the single DNA molecule level using nanofluidic channels

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SUPPLEMENTARY DATA

λ-DNA (48500 basepairs) was used as model DNA in this study. To justify the use of this model DNA, control experiments were performed with the actual phage P2 genome (34000 basepairs, GenBank ID: AF063097.1), in absence and presence of P2 Cox (Figure S1). The P2-DNA was pre-stained with YOYO as described in the main text for λ-DNA. Both genomes are compacted to a similar extent, slightly more for the P2 DNA, upon addition of P2 Cox.

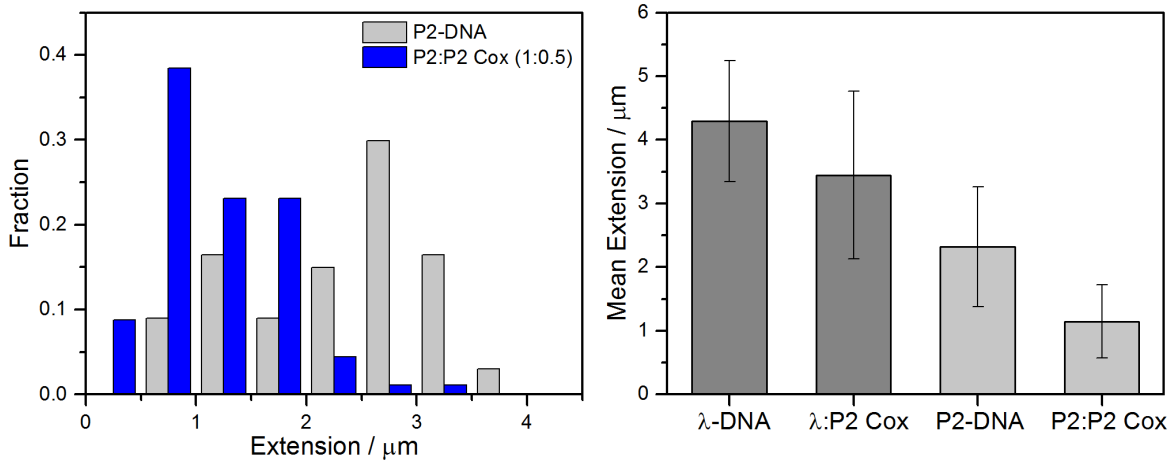

Figure S1. Extension of P2-DNA, confined to nanochannels of 200 nm width, in absence (grey) or presence of P2 Cox (blue) at a molar ratio of 1:0.5 (DNA basepairs:protein) (left). Mean values with standard deviations are presented to the right, data for λ-DNA are included. The molar ratio (DNA basepairs:protein) was 1:0.5.

The average extension of nanoconfined DNA was found to decrease in presence of Cox, indicating that the protein compacts the DNA, and more so with increasing protein concentration. Control experiments with further increased protein concentration were performed, showing that at protein concentrations above a binding ratio of 1:1 (DNA basepairs:protein) all complexes are short and hence strongly compacted (Figure S2). At the highest concentrations of P2 Cox (molar ratios 1:2 and 1:10) the molecular complexes were not only highly compacted (DNA appeared short in extension) but larger aggregates of (non-stained) protein onto the DNA might have formed, as judged by the difficulty to introduce the complexes into the nanochannels.

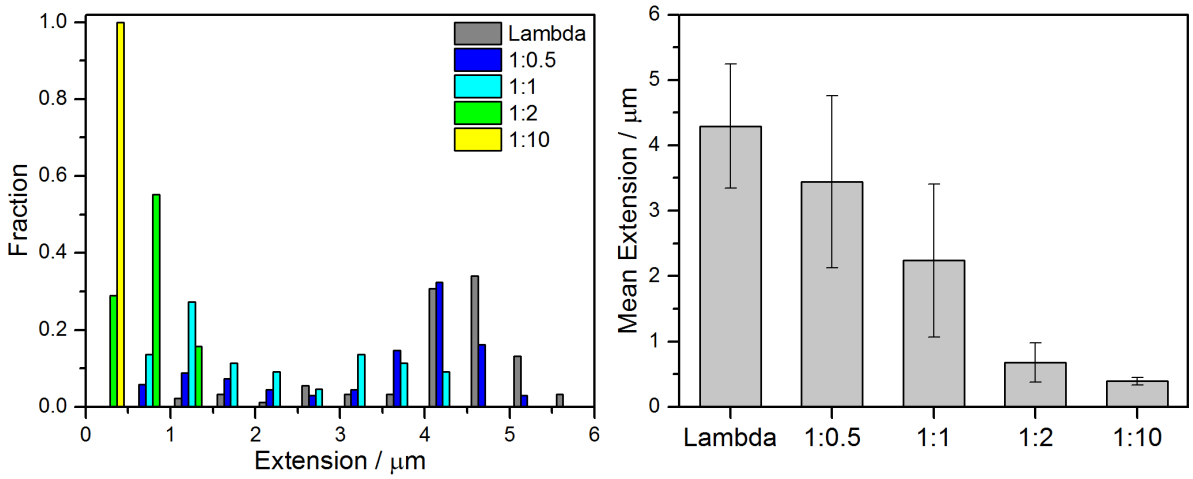

Figure S2. Extension of λ-DNA, confined to nanochannels of 200 nm width, in absence (grey) or presence of P2 Cox at indicated molar ratios (DNA basepairs:protein) (left). Mean values with standard deviations are presented to the right.

The emission intensity from the YOYO dye bound to the DNA-protein complexes was found to decrease with increasing DNA compaction, thus with increasing protein association to the DNA. At the highest degree of compaction there is still some dye bound, indicating that at least part of the DNA is exposed to the surrounding solution and supporting the hypothesis from our earlier work that the DNA is wrapped on the outside of the protein filament.[S1] As described in the Material and Methods section of the main text, the DNA was pre-stained with YOYO before mixing with protein. Control experiments were performed with the opposite mixing order, verifying the ability of YOYO to bind to Cox-bound DNA (Figure S3).

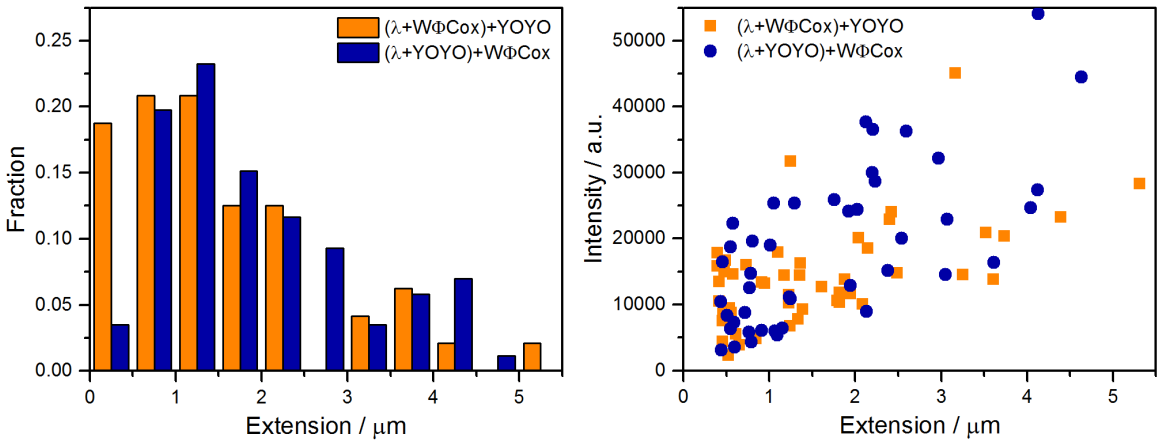

Figure S3. Extension (left) and emission intensity (right) of YOYO-stained λ-DNA, confined to nanochannels of 200 nm width, in presence of WΦ Cox. The DNA was either pre-stained before mixing with protein, as described in the main text, (blue) or incubated with protein at 30°C for 1 h prior to addition of YOYO and a further incubation at 30°C for 1 h (orange). The molar ratio (DNA basepairs:protein) was 1:0.5.

References

[S1] R. P. A. Berntsson, R. Odegrip, W. Sehlen, K. Skaar, L. M. Svensson, T. Massad, M. Hogbom, E. Haggard-Ljungquist, P. Stenmark, *Nucleic Acids Research* **2014**, *42*, 2725.